

A NOVEL INTEGRATED OPTICAL DISSOLVED OXYGEN SENSOR FOR CELL CULTURE AND MICRO TOTAL ANALYSIS SYSTEMS

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ABSTRACT

Production of accurate microscale oxygen sensors has been conventionally attempted using amperometric methods, which prove to be difficult to miniaturize, mainly due to the requirement of a bulky reference electrode. This paper details the design and fabrication process for an optically based oxygen sensor using an oxygen-sensitive fluorescent dye combined with microfabricated Cytop™ waveguides that are monolithically integrated into the sensing substrate. Application of the dye to the optical waveguides was accomplished using a unique electrostatic layer-by-layer deposition process that has allowed us to deposit ultrathin dye layers to specific areas of the substrate. Testing of the oxygen sensitivity of the immobilized dye was carried out.

INTRODUCTION

Despite recent advances in the field of biosensors, accurate microscale biosensing still proves to be difficult. Traditionally, oxygen sensors are based on amperometric type sensors, which, by consuming the oxygen content of the analyte in an electrochemical reaction, produce a small proportional current. However, these sensors suffer major drawbacks, such as biological material fouling, which limits the sensor life, the need for a complex reference electrode that limits the size of the sensor, and electrical interference susceptibility. Attempts have been made to miniaturize this type of sensor, with only limited results [1], due to the requirement of a reference electrode.

Optical sensing of biological compounds shows promise for overcoming the shortcomings of amperometric oxygen sensors, allowing high sensor resolution and selectivity, low biological fouling, minimal analyte interference, and the elimination of a reference probe. Oxygen naturally quenches the fluorescence emission of most common fluorophores, and also can be used as an intermediate analyte for other biochemical sensors, such as glucose. The dye tris(2,2'-bipyridyl dichlororuthenium) hexahydrate was chosen as the fluorophore, mainly for its selectivity and large Stokes shift [2]. This dye was immobilized on

specific sections of microfabricated optical waveguides that were integrated into a silicon substrate, thus allowing oxygen sensing to be carried out over discrete areas. Application of this sensor to the field of oxygen sensing encompasses both simple silicon wafer-integrated sensors and three-dimensional mapping of oxygen concentrations within artificial tissue engineered cell cultures, facilitated by integration of the waveguides into the cell substrates. Additionally, by replacing the oxygen-sensing dye with any number of analyte-specific fluorescence dyes, an almost limitless array of chemicals can be analyzed, such as sodium, potassium, carbon dioxide, several halides, and even some toxic chemicals such as sulfur dioxide. This flexibility opens application of this sensor to numerous biomedical fluid applications, but also to gas sensing, chemical reaction process monitoring and biological warfare agent detection.

FABRICATION OF INTEGRATED OPTICAL WAVEGUIDES

Fabrication of the monolithically integrated waveguides was accomplished by cutting channels into the silicon substrate using DRIE, coating with evaporated aluminum, and inserting Cytop™ amorphous fluoropolymer into the channels. The top of the Cytop™ waveguides was coated with sputtered aluminum and gold. This fabrication process is detailed in figure 1. Openings at the center sections of the fibers were made in the aluminum cladding of the fibers, to facilitate layering of the dye on these sections. Attachment of the input and output optical fibers was achieved using V-shaped grooves that were anisotropically etched using KOH etching into the substrate that allowed simple self-alignment of the optical fibers to the integrated waveguides. To interface the sample fluid with the integrated optical fiber, a channel was fabricated in a poly(methyl methacrylate) (PMMA) strip, and sealed to the waveguide substrate using poly(dimethyl siloxane) (PDMS). The channel was set up with fluidic input and output ports to facilitate dynamic, controlled dissolved oxygen testing of the completed sensor.

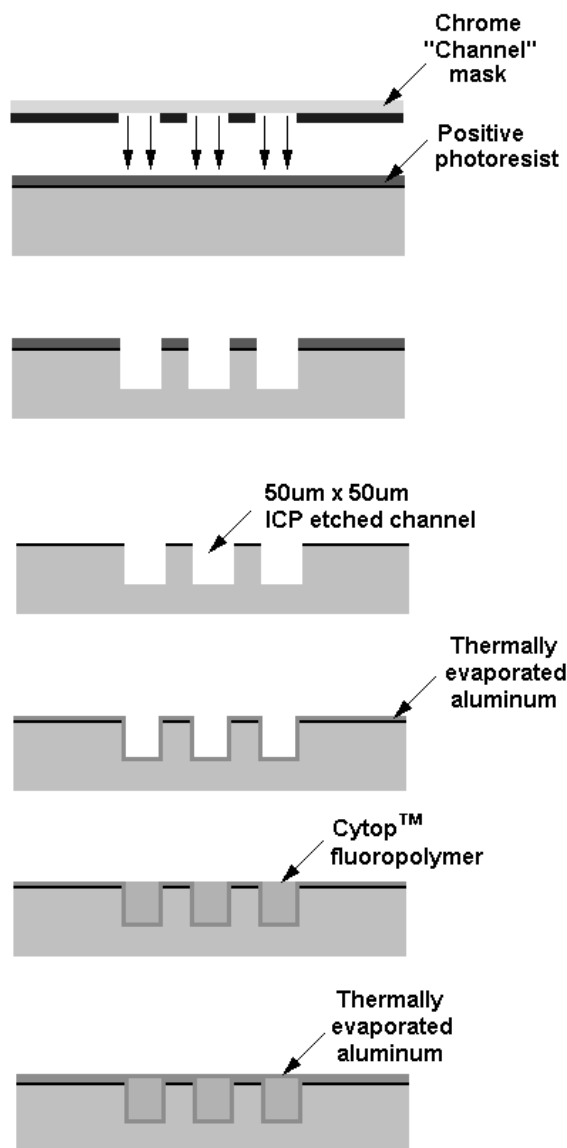


Figure 1. Waveguide fabrication

EVANESCENT WAVE INTERACTION

The light-fluorophore interaction in this oxygen biosensor takes place through the evanescent wave that occurs on the surface of the exposed waveguide. Using the evanescent wave penetration depth equation given as equation 1, the depth of the wave interaction was determined to be approximately 250nm.

$$d_p = \frac{\lambda / n_1}{2\pi[\sin^2 \theta - (n_2 / n_1)^2]^{1/2}} \quad (1)$$

DYE APPLICATION

Application of dye to the exposed waveguides was carried out using layer-by-layer self-assembly of the dye with positively and negatively charged polyions,

poly(sodium styrenesulfonate) (PSS) and poly(diallyl dimethylammonium) chloride (PDDA) respectively [3,4]. The polyions were applied in alternating layers, with layers of polyion adsorbing from solution to the charged substrate surface. Addition of a polyion layer caused a surface charge inversion of the substrate, thus facilitating the adsorption of further layers. The oxygen sensitive dye chosen, tris(2,2'-bipyridyl dichlororuthenium) hexahydrate, bears a relatively strong negative surface charge at neutral pH, and thus, when admixed with the polyion PSS, formed very stable interpolyelectrolyte complexes. These complexes, composed of ruthenium dye molecules surrounded by PSS strands, bore the same negative surface charge as PSS. When exposed to the substrate surface that had been prepared as positively charged with PDDA, these complexes adsorbed to the surface, thus effectively immobilizing the dye on the substrate, and inverting the surface charge. This process is detailed in figure 2.

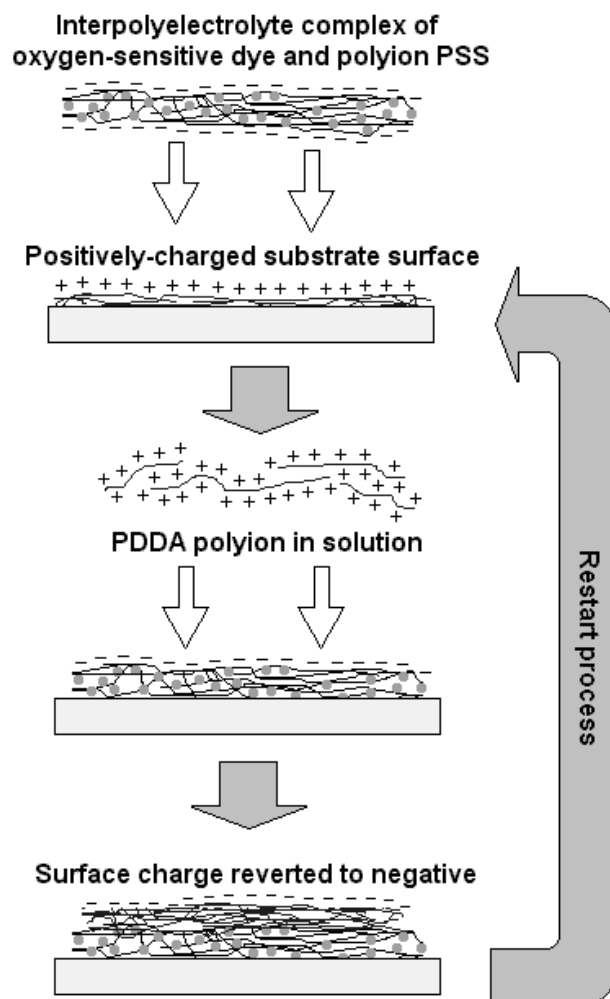


Figure 2. Dye layering process

To prepare the Cytop™ surface for dye layering, the wafer was activated using pure oxygen plasma to induce a negative charge on the exposed polymer surface. Five adhesion layers composed of PSS and PDDA were adsorbed to the waveguide surface in preparation for the dye layering adsorption process. A total of twenty dye/PSS layers were applied to the waveguides, for a grand total of 45 polyon monolayers. Upon completion of the dye layering process, the fluidic channel was fitted to the exposed waveguide surface. The hydrophobic interactions between the substrate and the PDMS coating on the PMMA fluid channel substrate was sufficient to provide low-pressure sealing of the channel. A controlled dissolved oxygen apparatus was constructed to calibrate the system, and preliminary oxygen sensitivity experiments were performed on the test glass substrates to test the functionality of the layered dye. A schematic of this apparatus is shown in figure 3.

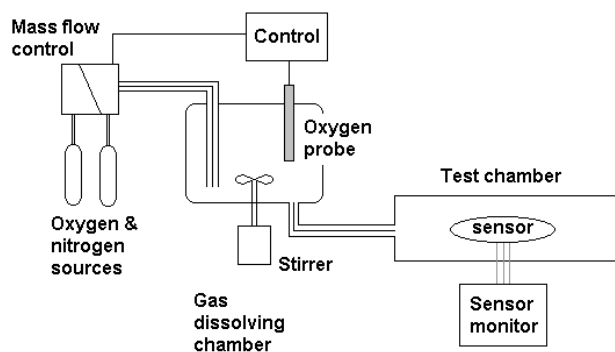


Figure 3. Controlled dissolved oxygen apparatus

DYE LAYERING RESULTS

Full characterization of the dye laying was carried out using quartz-crystal microbalance measurements, fluorescence tests and SEM analysis of the dye adlayers on glass test substrates. QCM analysis and SEM micrographs showed conclusively that efficient polyion adsorption was taking place.

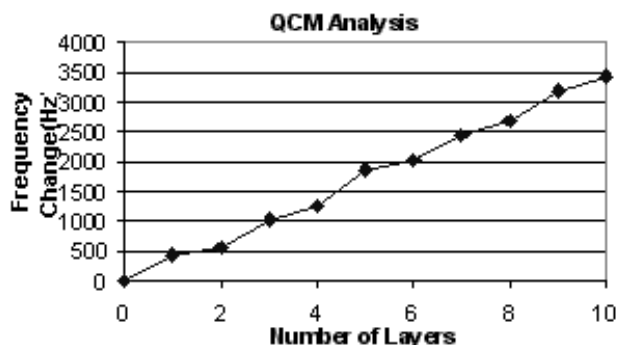


Figure 4. QCM dye-polyion layering results

The dye layer thickness was calculated using QCM data to be approximately 7.8 nm per dye-PSS monolayer, and 3.2 nm per PDDA monolayer. By simple calculation, the total thickness of the 45 layers is thus 249.8 nm, indicating that evanescent wave penetrates completely into the dye layer, thereby inducing optimal fluorescence. Fluorescence testing of consecutive dye-PSS adlayers demonstrated uniform predictable increases in emission intensity. This relationship is exhibited in Figure 6. Resolution of the system was calculated to be approximately 1 mg/L dissolved oxygen. Testing of successive adlayers was performed, and SEM photomicrographs show that the completed adlayers were very smooth.

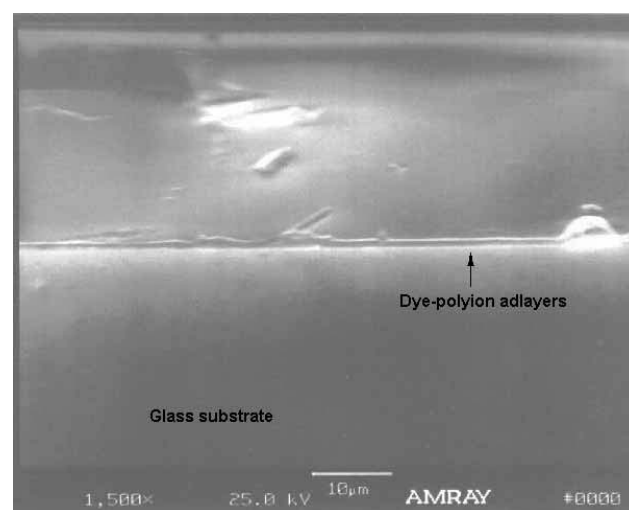


Figure 5. SEM of dye-polyion adlayers

The dye layers also demonstrated uniform surface coverage, even on imperfections in the glass substrate, and over trapped particles on the surface.

COMPLETED OXYGEN SENSOR LAYOUT AND TESTING

Figure 7 below shows the completed and assembled dissolved oxygen sensor, set up in a flow-sensing mode. Oxygen sensitivity over the range of 0mg/L to 36.8mg/L of dissolved oxygen was tested and the immobilized dye definitively demonstrated retention of oxygen sensitivity over the full experimental range as shown in Figure 8. While the system did not show linearity, it did show a clear trend and a fully repeatable signal. The curve fit to the data is also shown in Figure 8.

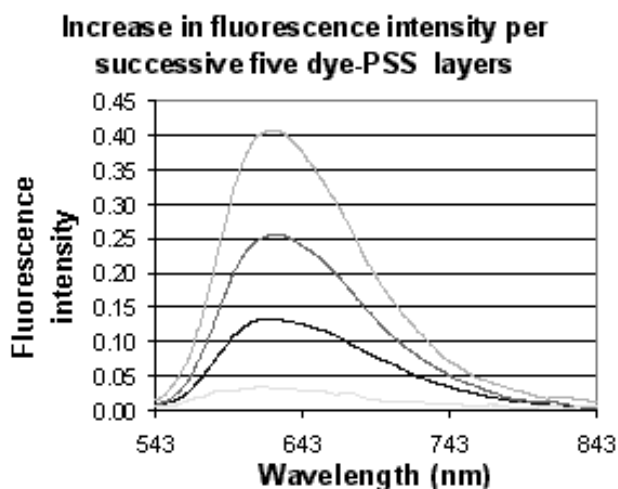


Figure 6. Fluorescence increase with successive groups of five dye-PSS layers

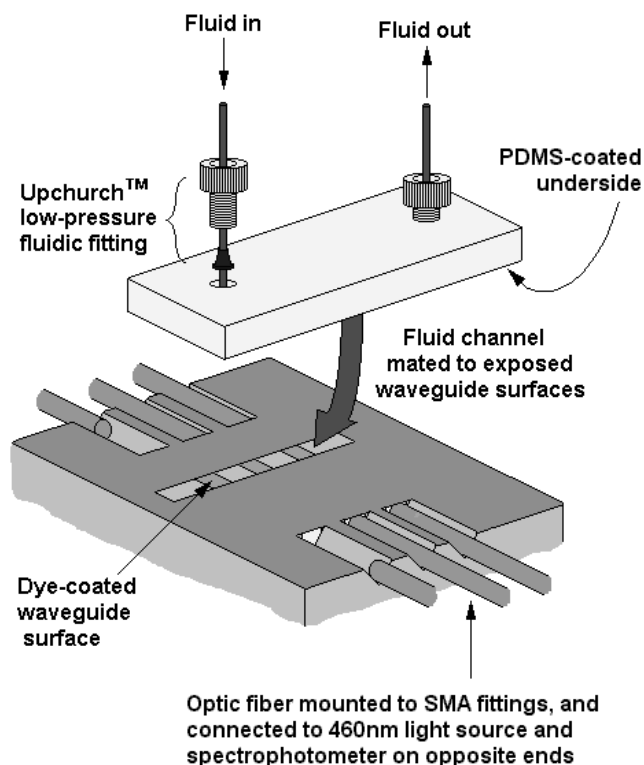


Figure 7. Completed dissolved oxygen sensor

DISCUSSION AND CONCLUSIONS

Integration of the nanoscale dye-polyion layers with the monolithic polymeric waveguides was achieved, and full oxygen sensitivity of the dye was maintained. The characteristic surface

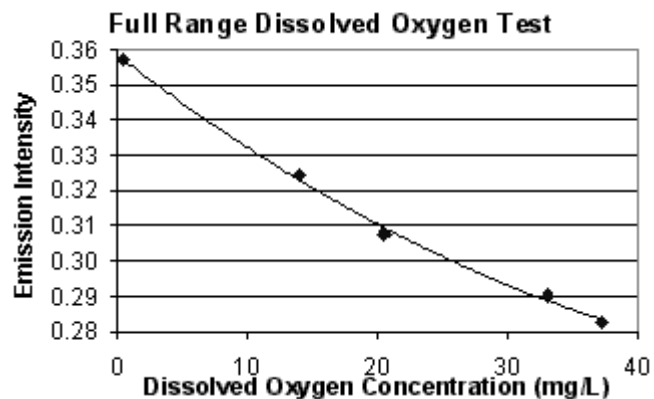


Figure 8. Full range dissolved oxygen test results

coverage of the polyion layers also lends to the feasibility of this dye immobilization methodology towards microfabricated tissue culture substrates, where surface morphology must be maintained. The polyion layers can also be modified to bear a surface more tenable to cell adhesion, simply by complexing the outer surface with an adhesion protein, such as fibrinogen. The general nature of this approach will also allow for a wide range of sensing arrays to be rapidly developed and fabricated. Controlled oxygen tests indicate the feasibility of this approach. The results also indicate that accurate calibration of this sensor is possible, and thus the overall viability of this sensor as a microscale oxygen sensor in a flow arrangement has been successfully demonstrated.

REFERENCES

- [1] Hermes, T. et al., "An amperometric microsensor array with 1024 individually addressable elements for two-dimensional concentration mapping", *Sensors and Actuators B Chemical*, 1994, pp. 33-37.
- [2] McEvoy, A. K., et al., "Development of a fibre-optic dissolved sensor based on quenching of a ruthenium complex entrapped in a porous sol-gel film", 1995, *SPIE*, vol. 2508, pp. 190-198.
- [3] Yoo, D., et al., "Investigations of new self assembled multiplayer thin films based on alternately adsorbed layers of poly electrolytes and functional dye molecules". *Materials Research Society Symposium Proceedings*, 1996, vol. 413.
- [4] Ariga, K., et al., "Assembling Alternate Dye-Polyion Molecular Films by Electrostatic Layer-by-Layer Adsorption", *Journal of the American Chemical Society*, 1997, vol. 119, pp. 2224-2231.